The Association of Negative Energy Balance, Sub-Clinical Hypocalcemia, and Periparturient Disease with Rate of Weight Loss and 30-Day Milk Production in Dairy Cattle

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Shanna Johnson
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Paula A. Ospina, Advisor

Abstract

The objective of this study was to analyze the association of abnormal blood metabolite levels (prepartum and postpartum non-esterified fatty acids (NEFAs), β-hydroxybutyrate (BHB), and calcium) and periparturient diseases (clinical ketosis, mastitis, displaced abomasum, retained placenta, metritis, lameness and periparturient paresis) with rate of weight loss and milk yield during a cow's first thirty days in milk. A total of 105 Holstein cows from three dairy farms in central New York State were analyzed in the study. Blood samples, back fat measurements, body condition scores, lameness assessments, and records for body weight, milk yield, and disease occurrence were collected at different time points beginning approximately a week before parturition until thirty days in milk. Back fat and body condition scores were not correlated with each other and were not included in the final analysis. The associations between the interaction of each blood metabolite with disease and change in body weight and milk yield were stratified by parity and evaluated with the MIXED procedure in statistical software. All cows which developed disease experienced a higher rate of weight loss and produced less milk than their healthy herd mates. Each parity group had a different indicator metabolite for predicting faster weight loss (parity 1 = prepartum NEFA, parity 2 = BHB, parity 3 = postpartum NEFA), but calcium was not a reliable indicator for any group. Elevated prepartum NEFA and BHB levels were associated with reduced milk production for cows of parity ≥ 2, while blood metabolites were only useful for heifers which concurrently developed disease. Through the use of these tests, farmers can intervene early to optimize animal health and economic return.

Acknowledgements

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Finally, I would like to thank my family, friends, and all of my other academic, research, and clinical mentors who have inspired me to be the person I am today through their unending encouragement and support. Because of them, I have accomplished much more than ever I thought I was capable of. They will continue to be my treasure and foundation as I pursue my professional goals!

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List of Abbreviations

BHB	β-hydroxybutyrate
DIM	Days in milk, calving date = Day 0
NEFA	Non-esterified fatty acid

Introduction

The contemporary dairy industry is of considerable interest to the economic health of the United States, generating an estimated $140 billion in economic output (Guduru, 2012) from 200 billion pounds of milk (Progressive Dairyman, 2013) made by over nine million cows (USDA/ National Agricultural Statistics Service, 2007). New York State ranks fourth nationally in total milk production, with about 610,000 cows and an average herd size of 118 (USDA, 2012). With this many animals in each herd, efficient management practices focus on groups; for example, cows are usually fed and housed by pen instead of by cow. As such, veterinary recommendations are focused on group or herd management.

To maximize economic return, individual productivity is still important; each cow represents a potential 22,000 pounds of milk yield per year (USDA, 2012). A successful lactation, characterized by high milk yield with minimal disease development, is essential to the economic sustainability of a dairy farmer. Therefore, identifying cows with a higher risk of poor economic return is helpful for balancing a producer's budget.

The objective of this study was to analyze the association of abnormal blood metabolite levels and periparturient diseases with rate of weight loss and milk yield during a cow's first thirty days in milk. High risk individuals are defined as those with circumstances that reduce their milk yield below that of their average healthy herd mate for the given lactation period. This classification also applies to cows which experience a more precipitous drop in body weight during the given lactation period and may be subject to a longer-term compromise in milk production and health outcomes.

The transition period was chosen as the time bracket of interest in analyzing the cows in this study. This period is characterized by a radical physical transition from gestation to lactation, and cows are challenged to adjusting to these dynamic physiological, metabolic, and nutritional shifts (Bauman and Currie, 1980). Cows which have difficulty in making a successful transition are subject to experiencing negative energy balance and hypocalcemia. According to Goff and Horst (1997), the transition period is also the time during which dairy cows experience the highest incidence of periparturient diseases, including but not limited to clinical ketosis, mastitis, displaced abomasum, retained placenta, metritis, lameness and hypocalcemia, which can be subdivided into sub-clinical and clinical presentation of periparturient paresis, known commonly as milk fever.

In this study, the change in body weight and milk yield over the cow's first thirty days in milk (DIM) were evaluated, with day zero = the calving date. The levels of non-esterified fatty acids (NEFAs) and β-hydroxybutyrate (BHB) in the cow's blood were measured as metabolic indicators. Serum calcium was used as an indicator of calcium homeostasis. The associations among these indicators, along with disease incidence, were analyzed with change in body weight and milk yield during the first 30 DIM as the outcomes. A BHB test strip currently costs $1.30 (mwivet.com), a local NEFA test costs $11.00 and serum calcium costs $8.00 (New York Animal Health Diagnostic Center).

The experimental goal is to compare the reliability of these tests with their cost to recommend to dairy farmers which blood metabolites are the most useful indicators of milk yield and body weight change during the first 30 DIM and when those readings should be collected.

Literature Review

The transition period between late gestation and early lactation, also known as the periparturient period, brackets the three weeks prior and following parturition in the dairy cow (Goff and Horst, 1997). This time is characterized by drastic physiological, metabolic, and nutritional changes to the cow's body as it adapts from carrying a fetus to producing copious amounts of milk. These shifts are the coordinated result of hormones and feedback mechanisms working to balance energy needs and maintain calcium homeostasis, described by Bauman and Currie (1980). During the transition period, these homeorhetic adaptations meet the challenges of doubling energy requirements (Bell, 1995) and calcium needs (Hansard et al., 1957; Martz et al., 1990) with a concurrent lag in dry matter intake (Drackley et al., 2001; Melendez and Risco, 2005).

In light of this, it is not surprising that most of the metabolic diseases of dairy cows occur during the first few weeks of lactation before peak milk yield (Goff and Horst, 1997; Ingvartsen, 2006). In addition to the immediate impact these disorders have on the cow's welfare, milk yield and reproductive performance for the current lactation, they can also burden the dairy producer with increased treatment costs and culling rates (Risco and Melendez, 2002). This is compounded by the fact that many of these calving-related diseases are associated with one another. For example, Curtis et al. (1983) reports that dairy cattle that develop milk fever are eight times more likely to develop mastitis during the same lactation, while LeBlanc et al. (2005) indicates that cows with sub-clinical ketosis are eight times more likely to develop a displaced abomasum. Melendez and Risco (2005) report on a large number of associations among periparturient diseases, linking periparturient paresis with retained placenta (odds ratio=2.0) and

metritis (=1.6) as well as clinical ketosis with periparturient paresis (=23.6) and displaced abomasum (=53.5). Both Duffield (2000) and Mulligan and Doherty (2008) further discuss the linked etiology of these periparturient diseases with one another and with the metabolic shifts the cow is experiencing at this time.

Negative Energy Balance

When a cow's metabolic output, in the forms of tissue maintenance, activity, and lactation, exceed the caloric intake from its feed, it is said to be in negative energy balance (Figure 1). This causes it to mobilize body reserves to compensate for the energy deficit between dry matter intake and the energy output for milk production (Bauman and Currie, 1980). Negative energy balance reaches its maximum one to two weeks after the onset of lactation (Butler and Smith, 1989), and disposes the cow to a host of metabolic disorders such as fatty liver and ketosis (Doherty, 2002; Bertics et al., 1992), displaced abomasum (Duffield et al. 2009; LeBlanc et al., 2005), retained placenta (Cameron, 1998), and immunosuppression (Goff, 2008).

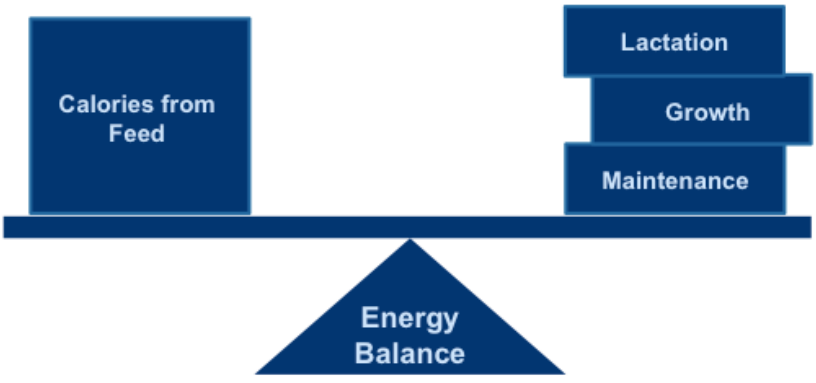

Figure 1. Energy balance. A cow's energy status represents the dynamic balance between caloric inputs and outputs. If a cow expends more energy than it can obtain from its feed, this equilibrium "tips" to the right and it is considered to be in a state of negative energy balance.

Etiology and Impact of Negative Energy Balance

The cow's glucose requirements spike from 1 kg/day during late gestation to over 2.5 kg/ day during the postpartum transition period (Reynolds et al., 2003). Because little glucose is absorbed from the gut (Otchere, 1974), two key adaptations to this accelerated energy demand are mobilization of triglycerides in adipose tissue to non-esterified fatty acids (McNamara, 1991) and hepatic oxidation of circulating NEFAs to β-hydroxybutyrate (Emery et al., 1992). This, in addition to an increase in gluconeogenesis from amino acid, lactate, and glycerol precursors provides the necessary energy to support the mammary gland (Drackley, 1999; Herdt, 2000; Reynolds, 2003) for the production of lactose (Bruss, 2008; Figure 2).

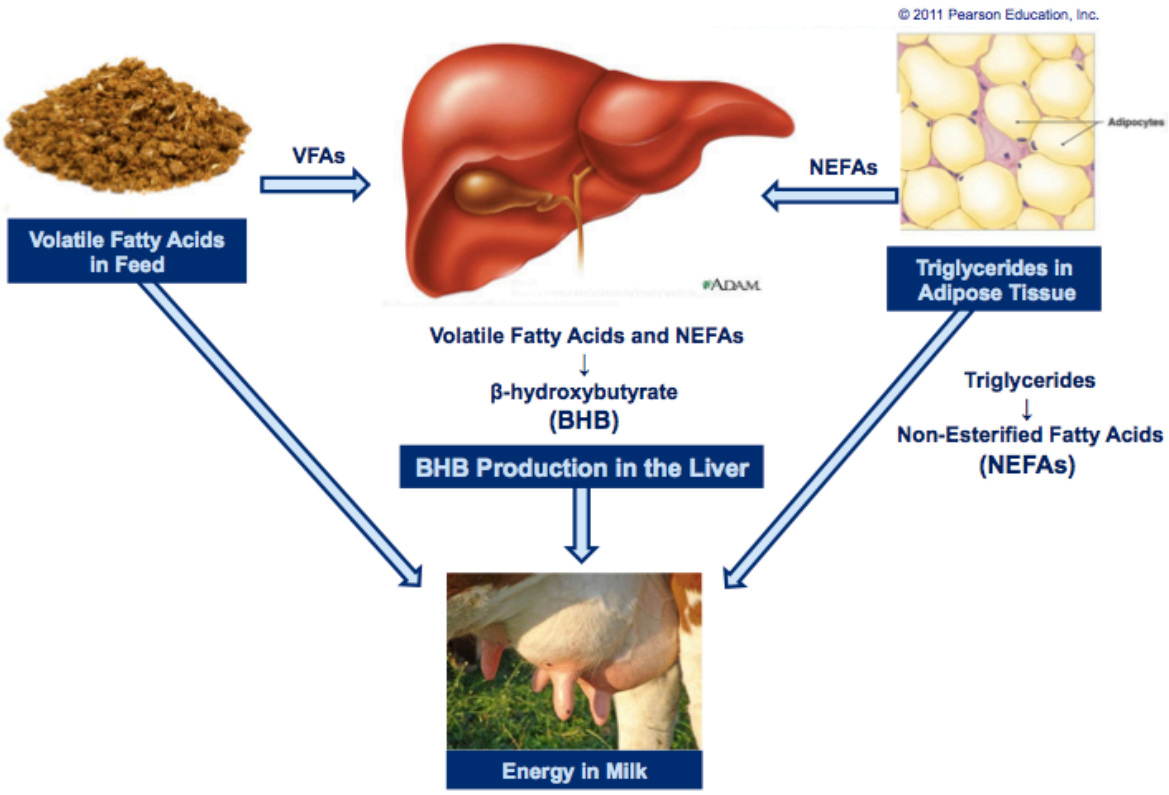

Figure 2. Blood metabolites and the biology of energy balance. The energy necessary for lactation comes from a variety of sources, as elaborated upon by Bruss (2008) and Drackley et al. (2001).

If the production of NEFAs and BHBs exceed the ability of the mammary gland to metabolize them for energy, they accumulate in the bloodstream (Adewuyi, 2005). Clinically ketotic cows experience reduced dry matter intake, a loss in milk yield, and demonstrate symptoms of nervous system involvement such as staggering, lack of coordination, and blindness (Adewuyi et al., 2005). Even sub-clinical levels of NEFAs and BHB are a detriment to the cow because they make it almost tenfold more likely to develop clinical ketosis (Suthar et al., 2013), as well as predisposing it to developing a displaced abomasum, mastitis, metritis, and lameness (Lean, 2011). Furthermore, both severity and duration of high BHB levels can interfere with reproductive success (Walsh et al., 2007).

These effects are most pronounced immediately after calving, when blood NEFA and BHB levels are highest (Weber et al., 2013). Ospina et al. (2010a) determined critical thresholds for blood NEFA and BHB levels associated with the onset of periparturient diseases up to 120 DIM. McArt et al. (2012) and Gröhn et al. (1998) reported that cows above the $\geq$ 1.2 mmol/L threshold for BHB concentrations were more likely to be culled from the herd, costing dairy producers about $145/case (Melendez and Risco, 2005).

Quantifying Energy Balance

While the relationship between these diagnostic parameters and milk yield has been well-studied (Dohoo and Martin, 1984; Duffield et al., 2009; Lean, 2011; Ospina et al., 2010b and 2010c), their association with change in body condition over time is not well understood. This can be studied objectively using changes in body weight over time, which according to Kohiruimaki et al. (2006) is a reliable indicator for the anticipated incidence in periparturient

disease. The drawback is that this does not representatively account for the impact a given weight loss value has for cows of different starting weights.

Another option is to use the body condition scoring system developed by Edmondson et al. (1989), which is a subjective assessment of the cow's fat reserves through visual assessment of its lipid stores relative to its body frame (Figure 3). Studies link body condition score to milk yield in dairy cows up to 120 DIM (Domecq et al., 1997). This has the inherent disadvantage of different score assignments by different individuals, although observer agreement within a quarter point standard deviation was about 90% in a survey by Ferguson et al. (1994). Its utility in accurately predicting body weight when hip height and hip width are provided in a mathematical model was supported by Enevoldsen and Kristenen (1997), and Waltner et al. (1994) testifies that combining body condition score and body weight reliably predicts body fat content in lactating dairy cows.

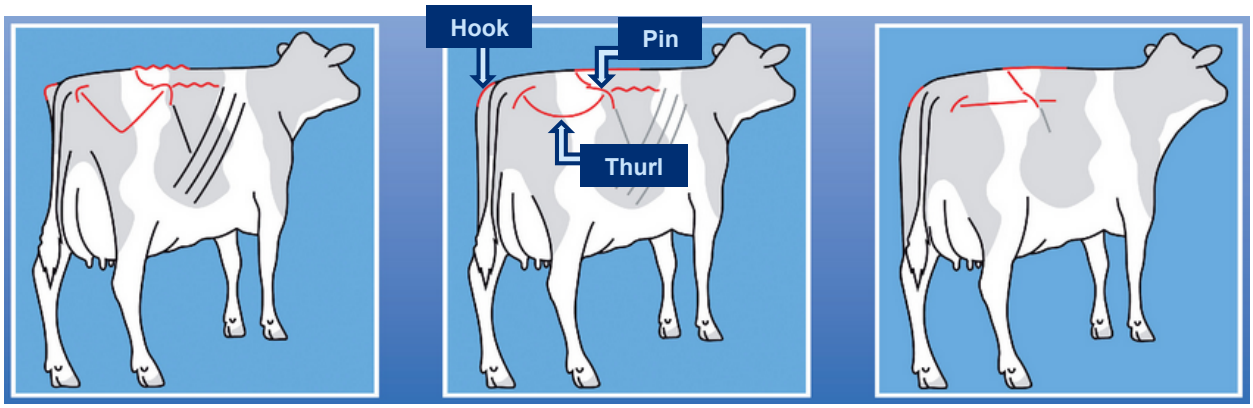

Figure 3. Determination of body condition score based on visual assessment. Body condition scoring is an objective assessment of a cow's energy status by evaluating the degree of adiposity on the cow relative to its body frame, using anatomical landmarks such as the hook, pin, and thurl. This image was modified from BCS by Deutscher Holstein Verband e.V. [Internet] http://www.holstein-dhv.de/exterieur.html. Accessed 30 March 2013.

A final criterion is the use of diagnostic ultrasound in screening the back fat thickness of these cows, which can serve as an objective test of the degree of adipose tissue mobilization at the level of herd screening (Schröder and Staufenbiel, 2006).

Calcium Homeostasis

Maintaining constant blood calcium levels, at the narrow range of eight to ten mg/dL in dairy cows (Goff et al., 1996) is critical to neuromuscular excitability, blood clotting, and endocrine function (Griffin and Ojeda, 1996). According to DeGaris and Lean (2008), the lactating dairy cow can lose up to 50 grams of calcium to mammary production on a daily basis, which equates a four- to five-fold increase of plasma clearance relative to the rate at parturition (Oetzel and Goff, 1998). The cow's options for meeting these calcium needs are limited to increasing intestinal absorption of dietary calcium, and mobilizing calcium from skeletal reserves following parathyroid hormone signaling (DeGaris and Lean, 2008; Goff, 2008, Figure 4).

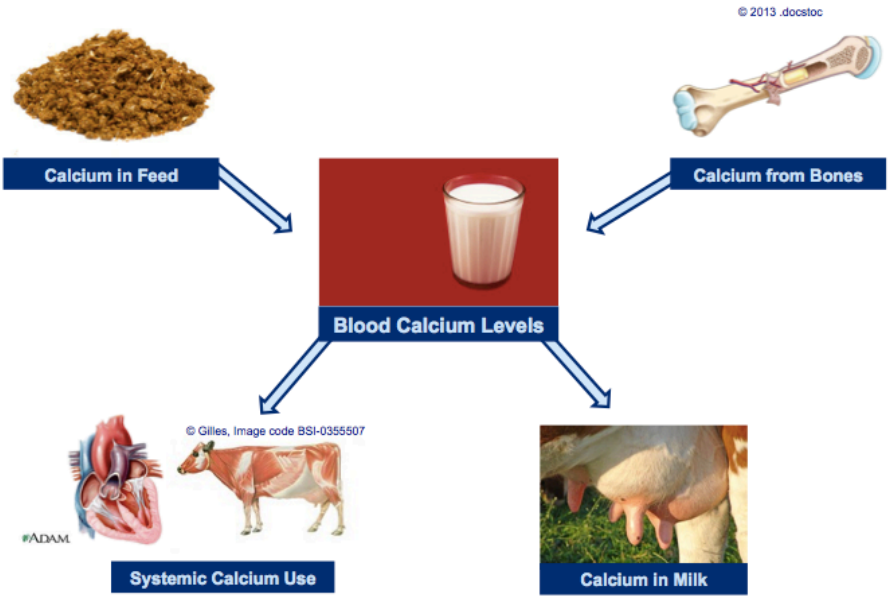

Figure 4. The biology of calcium homeostasis. To meet the calcium demands imposed by lactation while maintaining systemic levels for homeostasis, the dairy cow obtains calcium from its feed and skeletal reserves, as discussed by DeGaris and Lean (2008) and Goff (2008).

Etiology and Impact of Hypocalcemia

When the cow is unable to restore homeostatic blood calcium levels and they drop below 5.6 mg/dL (Degaris and Lean, 2008), it experiences the neuromuscular dysfunction typical to milk fever, known clinically as periparturient paresis (El-Samad et al., 2002; Horst et al., 1997). Upon manifestation of these symptoms, intravenous calcium is therapeutically administered to sustain the cow until its intestinal absorption and bone reabsorption can adapt, without which up to 70% of clinically affected cattle can be culled (Horst et al., 1997). Between six and eight mg/dL, the cow still experiences reduced feed intake, poor rumen motility, reduced milk yield, and the increased incidence of disease (Chapinal et. al, 2011; Horst et al., 2003; Ingvartsen, 2006; Klerx and Smolders, 1997). This could be due in part to sub-clinical hypocalcemia handicapping the ability of neutrophils to phagocytize pathogens (Martinez et al., 2012), decreasing immune cell sensitivity to pathogenic stimuli (Kimura et al., 2006), and exacerbating periparturient immunosuppression with lowered plasma cortisol levels (Goff and Horst, 1997), opening the door for infectious diseases such as mastitis and metritis.

Low serum calcium concentrations are also associated with negative energy balance (Reinhardt et al., 2011). As Daniel (1983) described, serum calcium concentrations below 5 mg/dL can reduce abomasal motility by 70% and the strength of contraction up to 50%, which inhibits rumen function. Consequently, dry matter intake decreases, with a parallel rise in the severity of negative energy balance. Grummer (1996) brought this process full-circle in emphasizing that increasing blood NEFA concentrations can further suppress appetite.

Reinhardt et al. (2011) determined the annual incidence of periparturient paresis in the United States to be around 1% for heifers and 6% for second and greater lactation dairy cows, while Horst et al. (2003) reports that sub-clinical hypocalcemia can affect up to 50% of older

cows. Milk fever poses a notable economic burden to dairy producers because of reduced milk yield (Østergaard and Gröhn, 1999), costly intervention therapies (Kossaibati and Esslemont, 1997), and associations with other diseases (Curtis et al., 1983; Fleischer et al., 2001; Thirunavukkarasu et al., 2010).

Common Diseases of the Periparturient Period

Periparturient diseases in dairy cattle often arise as a consequence of accelerated energy and calcium demands during the transition period. While the term was traditionally applied in reference to metabolic disorders, its definition has since expanded to include other diseases that commonly occur around calving, such as retained placenta, displaced abomasum, and mastitis (Mulligan and Doherty, 2008). While their clinical manifestations were not the focus of this study, the substantial economic consequences this poses to the dairy producer (Kelton et al., 1998; Melendez and Risco, 2005) justifies evaluating them in terms of making practical recommendations to the dairy farmer.

Displaced abomasum

Displaced abomasum is clinically defined as the abomasum filling with gas and rising up along the side of the rumen, causing a loss in appetite and the production of a high-pitched "pinging" sound upon percussion of the abdominal wall (Kelton et al., 1998). Its incidence in dairy herds is between 1.7% and 3.3% (Ingvartsen, 2006). Melenez and Risco (2005) quantified the economic losses due to displaced abomasum to be about $340 per case, and between 250 and 2000 kg of milk per lactation.

Risk factors for developing a displaced abomasum include prepartum negative energy balance indicated by high serum NEFA and BHB concentrations, consuming high energy feeds,

calving in the winter, birthing twins, development of retained placenta and/or metritis, (Cameron et al., 1998; LeBlanc et al., 2005). The role of hypocalcemia in the manifestation of displaced abomasum is currently unclear (Curtis et al., 1983 and Leblanc et al., 2005). Low parity and having a body condition score above 4.0 been implicated as a risk factor for displaced abomasum development (Cameron et al., 1998 and Shirley, 1994, respectively), although their utility as predictors is still questioned (Dohoo and Martin, 1984; Leblanc et al., 2005). What is clear is that a reduction in abomasal motility is a key step towards displaced abomasum development. Ketosis is often associated with a low dry matter intake, causing reduced fluid volume and limiting forestomach motility, the effects of which are passed on to the abomasum (Cameron et al., 1998).

Lameness

According to the visual locomotion scoring protocol recommended by Bicalho et al. (2007), a cow is clinically lame if its ambulation score is ≥ 3 during the first 70 DIM. This is often accompanied by distinct favoritism in weight-bearing limbs. It affects 7% to 14% of dairy cattle (Ingvartsen, 2006) and mostly occurs early in lactation even though most physical injuries to the hoof occur prepartum (Wilde, 2006). Lameness exacerbates the metabolic impacts of negative energy balance by reducing the cow's willingness to regularly feed, thereby lowering dry matter intake. Economic losses are a product of reduced milk yield (Warnick et al., 2001) and increased risk of culling (Esslemont and Kossaibati, 1997; Sprecher et al., 1997). Lameness causes dairy producers about $300 per case, according to Melendez and Risco (2005). Risk factors for the development of lameness include laminitis, negative energy balance, and a loss in body condition score (Collard et al., 2000; Wells et al., 1993).

Mastitis

Kelton et al. (2008) characterized clinical mastitis as an infection involving the secretion of visually abnormal milk (watery or containing clots of flakes) from one or more quarters, often accompanied by inflammation and edema of the udder. Rajala-Schultz et al. (1999) reports that milk losses from mastitis throughout a lactation can tally between 100 and 500 kgs of milk, and that cows affected by mastitis did not recover their premastitis milk yield throughout the course of the study. The incidence of clinical mastitis is typically between 14% and 17% (Ingvartsen, 2006) but it can affect up to 50% of a dairy herd (Melendez and Risco, 2005). Risk factors for the development of mastitis include being a Holstein-Fresian cow, high milk yield, hypocalcemia, and negative energy balance (Berry et al., 2007; Curtis et al., 1983; Ingvartsen et al., 2003; Mulligan and Doherty, 2008). Berry et al. (2007) reported a negative association between body weight and masitis in first parity cows, but this relationship was reversed for second lactation and greater cows.

Retained Placenta and Metritis

Retained placenta is defined as the failure of fetal membrane expulsion during the final stage of parturition, identified by the presence of fetal membranes at the vulva or in the uterus or vagina more than 24 hours after the first postpartum observation (Kelton et al., 1998). This predisposes the cow to developing metritis, the diagnosis of which requires abnormal cervical discharge, vaginal discharge or both or uterine content often accompanied by an elevated temperature and reduced dry matter intake (Kelton et al., 1998; Mulligan and Doherty, 2008). Retained placenta is seen in 7% to 9% of dairy cows, while 10% to 13% experience metritis (Ingvartsen, 2006). These disorders take an economic impact to the farmer in terms of treatments for infection, greater number of days from calving to conception, and culling

(Melendez and Risco, 2005). These monetary losses from metritis total about $106 per lactation in 1986 (Bartlett et al.), while the price tag of retained placenta was set at about $285 in 2005 (Melendez and Risco).

Cows on their third lactation or greater experience retained placenta about twice as often as heifers and second lactation cows (Bartlett et al., 1986). Other risk factors include high prepartum NEFA levels and hypocalcemia (Curtis et al., 1983; Dubuc et al., 2010; Wilde, 2006). Forty-four percent of cows that had retained placenta develop metritis over during their current lactation, versus sixteen percent of cows which were not affected with retained placenta but developed metritis. This causal relationship has been investigated in other studies as well (Dohoo and Martin, 1984; Dubuc et al., 2010; Fleischer et al., 2001).

Current Shortcomings

Although the associations among negative energy balance, calcium homeostasis, and both disease and milk production in the transition period have been comprehensively studied, some deficiencies in knowledge persist. Few studies simultaneously collected information on levels of NEFAs, BHB, and calcium, daily body weight and daily milk production for the purposes of evaluating a dairy cow's whole health status during the early lactation period.

Collard et al. (2000) evaluated body weight on a weekly, monthly, or every 100 day-basis and found that it is an important indicator of future lactation success and disease risk. However, body weight cannot be measured easily nor frequently on conventional farms with limited weight detection capabilities. With the advent of automatic milking systems that employ built-in weight scales, body weight can be regularly monitored as an index of health. While previous studies have investigated the links between these metabolic indicators and milk yield, (Chapinal et al.,

2012; Jawor et al., 2012; Østergaard and Larsen, 2000), their associations with changes in body weight throughout lactation have not been as thoroughly explored. In a recent study, Weber et al. (2013) reported that cows with the highest NEFA values demonstrated the greatest loss in body weight, while those with moderate NEFA levels lost more back fat. However, this study did not examine the relationship between blood BHB or serum calcium levels and body weight. The current study aimed to address this lack of information.

Materials and Methods

Study Population

Holstein cows, a breed of domestic cattle *Bos taurus*, were studied from three conveniently-selected commercial farms in central New York State. Inclusion criteria for these herds were as follows: 1) >100 milking cows, 2) free-stall housing, 3) fed a total mixed ration formulated to meet or exceed the National Research Council nutrient requirements for lactating Holsteins according to farm conditions, and 4) use of automatic milking systems (Astronaut A3 and A4, Lely Industries N.V, Rotterdam, The Netherlands). All farms consented to participate, and the study was approved by the Institutional Animal Care and Use Committee.

From these herds, a convenience sample of heifers and cows calving in between June 11th and August 18th of 2012 were enrolled in the study. The goal was to enroll 100 cows and follow them until 30 DIM in order to find a minimum of 1.5 kilograms of difference in daily milk production between groups, with a 3 kilogram standard deviation, 95% confidence interval and power of 80%. Both nulliparous heifers and multiparous cows were enrolled in the study. Enrollment in the study occurred once weekly between three and 10 days prior to the expected calving date predicted by each herd's farm management software.

Data Collection

During enrollment, blood was collected from the coccygeal vessels from each cow expected to freshen in the following week into a 10-mL non-additive evacuated glass tube using a 20-gauge, 2.54-cm blood collection needle. The BHB concentrations were evaluated immediately cow-side using the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL; Iwersen et al., 2009). Within 30 minutes of collection, all blood samples were centrifuged for 15 minutes at 2,000xG. Following this, serum was harvested and stored in ice for transportation to

the lab, where it was frozen at -20°C according to the recommendations of Stokol et al. (2005). All serum samples were then sent to New York Animal Health Diagnostic Center (Ithaca, NY) for total calcium concentration (Roche Diagnostic reagents) and serum NEFA concentration analysis (NEFA-C, Wako Chemicals USA Inc., Richmond, VA). In addition to blood sampling, enrolled cows also had their body condition score determined on a 5-point scale (1=emaciated, 5=obese, scored in 0.25-point intervals) using the method described by Ferguson et al. in 1994 (Figure 3). Lameness was also assessed visually on a 5-point scale (1=normal; 3=moderately lame, cow clearly favored one or more limbs; 5=severely lame, non-weight bearing, scored on whole number intervals) according to the standards set by Bicalho et al. (2007; Figure 5).

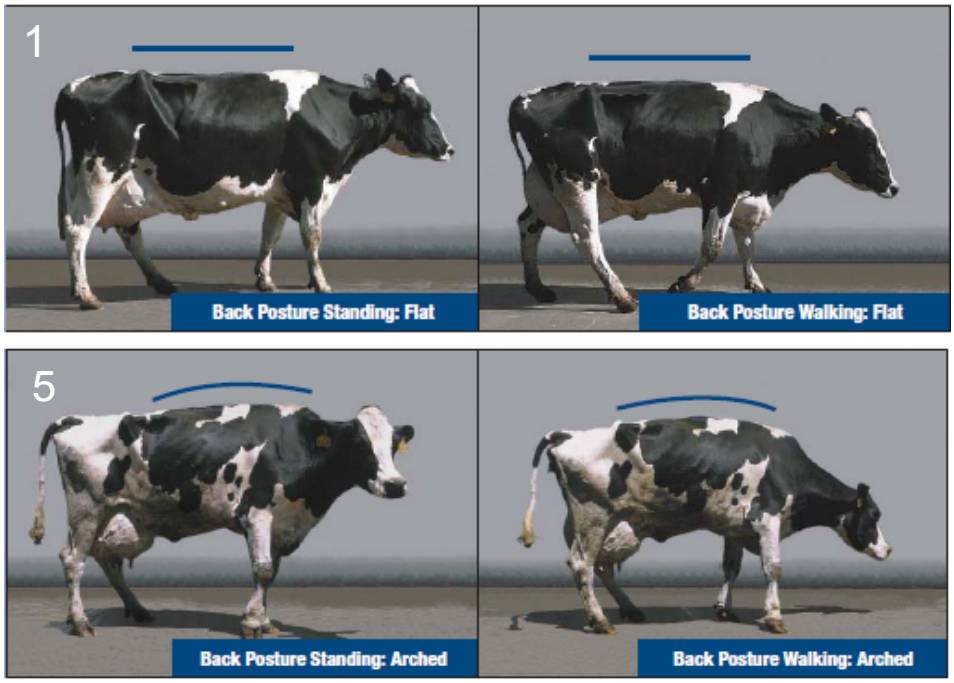

Figure 5. Determination of lameness score based on visual assessment. Scoring is performed by veterinarians and herdsmen alike as part of a routine physical exam. This image was modified from Locomotion Scoring of Dairy Cattle by Dr. Steven Berry. University of Wisconsin-Madison and Zinpro Corporation. Published 5 March 2005. [Internet] http://www.vetmed.wisc.edu/dms/fapm/fapmtools/6lame/New5point_locomotionscoreguide.pdf. Accessed 30 March 2013.

Back fat thickness was evaluated using an Ibex Pro portable ultrasound machine with an 8.5MHz 66-mm linear probe (E.I. Medical Imaging, Loveland, CO) to the nearest millimeter in the manner described by Schröder and Staufenbiel in (2006; Figure 6).

Figure 6. Determination of back fat thickness using a portable ultrasound machine. Back fat thickness is measured to the nearest millimeter using a portable ultrasound machine with a linear probe. According to the method set by Schröder and Staufenbiel in 2006, the superficial termination is the skin and the deep termination is the fascia trunci profunda.

After each cow was enrolled, blood samples were collected multiple times: at one, two, three, and five days postpartum, then weekly until 30 DIM. Serum NEFA was evaluated one week prepartum and at five DIM; whole blood BHB was evaluated one week prepartum, at three and five DIM, and weekly until 30 DIM; serum total calcium was evaluated one week prepartum, and at one, three, and five DIM; body condition score and back fat were evaluated

one week prepartum, at five DIM, and weekly until 30 DIM, and lameness was evaluated one week prepartum and weekly until 30 DIM (Table 1). Back fat and body condition score were evaluated at day five, then weekly until 30 DIM along with lameness scores. Daily body weight and daily milk weight for each cow was collected by the Lely T4C management system (Lely Industries N.V/, Rotterdam, The Netherlands).

	-1 Week	+1 Day	+2 Days	+3 Days	+5 Days	Weekly until 30 DIM
NEFA	×				×	
BHB	×			×	×	×
Total Calcium	×	×	×	×	×	
Back Fat	×				×	×
Body Condition Score	×				×	×
Lameness	×					×

Table 1. A complete sample collection schedule of blood chemistry, body condition score, back fat, and lameness. All days listed are relative to calving date (0 DIM).

Farm personnel also documented the occurrence of clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and metritis on either Dairy Comp 305 (Valley Ag. Software, 2009) for two farms, or paper records for the third farm. Standard disease definitions were discussed with the workers at each farm at the start of the study, however the diagnosis and care of affected animals was delivered according to farm protocols defined by the veterinarian responsible for the herd.

Statistical Analysis

The daily body weight and daily milk weight information was imported from the Lely T4C management system (Lely Industries N.V/, Rotterdam, The Netherlands) into Excel (Microsoft, v. 97-2003). The slope of the change in body weight for each animal during the first 30 DIM was estimated with simple linear regression with a line of best fit (JMP statistical package, version 9 SAS Inst. Inc., Cary, NC, 2011). The sum of milk production over the first 30 DIM was also evaluated. For cows missing only one or two data points, missing values for daily milk weights were filled-in using the linear regression line of best fit equation determined by the same JMP procedure used for body weight change.

Statistical Summary

Descriptive statistics were generated with the FREQ and MEANS procedures of SAS (version 9.3 SAS Inst. Inc., Cary, NC). This was used to characterize the data. The CORR procedure, specifically Pearson's correlation coefficient, was used to evaluate the correlation between back fat and body condition score. The MIXED procedure was used to evaluate the association between each blood metabolite with change in body weight and milk production during the first 30 DIM. These univariable analyses were used to determine which sample among metabolites that were measured on more than one day (BHB and calcium) would be used in the final analyses. The predictors included in the final analyses for the change in body weight over time and milk production during the first 30 DIM were parity group, disease development, dichotomized levels of pre- and post-partum NEFA, BHB, and calcium with herd treated as a random effect.

Based on information from previous reports which associate certain concentrations of calcium, NEFA, and BHB with negative downstream outcomes (Ospina et al., 2010a; Reinhardt

et al., 2011), the total calcium concentrations were dichotomized at ≥ 6.0 mg/dL to 8.0 mg/dL to evaluate the effect of sub-clinical hypocalcemia, pre-partum NEFA was dichotomized at ≥ 0.3 mEq/L; post-partum NEFA ≥ 0.7 mEq/L, and BHB at ≥ 1.2 mmol/L.

Stratification by Parity

The difference in mean change in body weight and total milk production in the first 30 DIM by parity group (parity =1, parity =2, parity ≥2, and parity ≥3) was evaluated using the T-test procedure in SAS. Analyses were stratified by parity if there was a significant difference ($p < 0.05$) between parity groups in milk production or body weight. Three lactation groups were evaluated separately in the body weight model: parity =1, parity =2, and parity ≥ 3. Two lactation groups were evaluated separately in the milk production model: parity =1 and parity ≥ 2.

Metabolite Analyses, Disease, and Interactions as Covariates

The effect of these metabolites on body weight change and total milk yield over the first 30 DIM were each evaluated in separate univariable models. Calcium and BHB results were screened for the best predictive sample, and prepartum and postpartum NEFA measurements were treated as unique predictors. While controlling for the effect of other metabolite samples collected on different days, the measurement that resulted in the smallest p-value and had the largest estimate difference from a baseline of zero was defined as the best predictive sample and kept in the final model. Because the effect of the various indicator metabolites on body weight change and milk yield outcomes was different ($p < 0.01$) based on the presence or absence of disease (clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and/or metritis), the interaction between each indicator metabolite and disease was forced into the final model.

The final model consisted of the best predictive metabolite, the effect of disease occurrence, and the interaction between disease and the metabolite with herd being treated as a random effect. The occurrence of any disease was categorized as 1 if the animal developed any of the following diseases: clinical ketosis, displaced abomasum, lameness, mastitis, retained placenta, or metritis during the first 30 DIM. The MIXED procedure was used to evaluate the association between interactions of blood metabolites and disease. The Least Squares Means estimates and p-values were reported for the interactions and they evaluate whether the combination estimate is different from zero. However, these combination estimates were also compared to each other using the DIFF statement of the Least Squares Means model in SAS, and superscripts denoting a statistical difference of $p > 0.05$ were also noted.

Results

Study Population Descriptive Results

A total of 114 cows were enrolled in the study, with nine cows excluded from data analysis due to removal from the herd resulting in fewer than 30 DIM of body weight and milk yield information. Of the nine cows excluded, three died (one listeria, one back injury, and one undefined), two were culled, and four had an incomplete data set due to technical difficulties with the automatic milking systems, bringing the final sample population for this study to 105 cows. Overall, these cows averaged 34 - 36.1 kg of milk production per day, and 1023 - 1083 kg of milk production total during the first 30 DIM.

Descriptive frequencies of the study population are presented in Table 2, and are organized by parity, blood metabolite groups and whether or not they had disease (clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and metritis). The frequency of each disease condition by parity is shown in Figures 7 and 8. Back fat thickness was weakly correlated with body condition score: R^2 for weeks one through three was 0.3 and for week four was 0.7.

Parity	High NEFA[a]	High BHB[b]	Sub-clinical [+ Clinical Hypocalcemia] [c]	Disease [d]	Total in Each Parity
1st	19	7	6	16	30
2nd	18	21	16	16	28
3rd+	22	29	32 [+3]	50	42
Total	59	57	54 [+3]	82	105
Percent Total	56.2%	54.3%	51.4% [2.9%]	78.1%	

Table 2. Number of cows grouped by parity, dichotomized by blood metabolite groups, and dichotomized by disease occurrence in 105 Holstein cows within the first 30 DIM.

[a] A prepartum NEFA of $\geq$ 0.3 mEq/dL and/or a postpartum NEFA of $\geq$ 0.7 mEq/dL.

[b] Any BHB reading $\geq$ 1.2 mmol/L.

[c] Any calcium reading of 6 – 8 mg/dL (sub-clinical) or < 6 mg/dL (clinical).

[d] Diseases include clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and/or metritis. Note that each condition is not mutually exclusive.

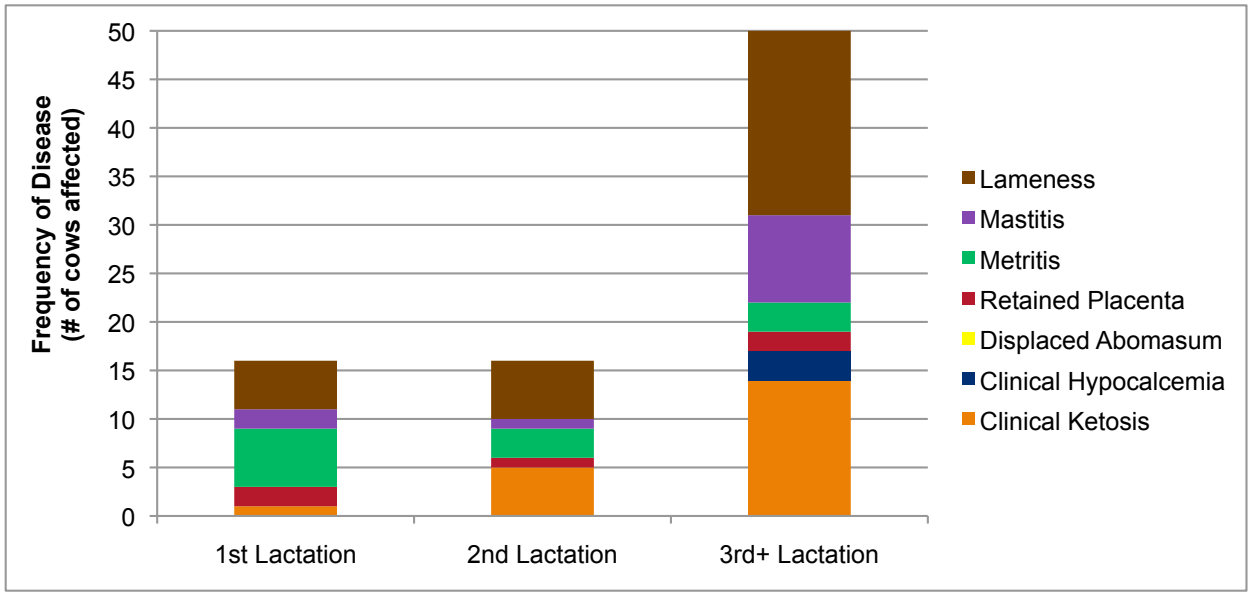

Figure 7. The frequency and distribution of disease conditions (clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and/or metritis) among the study population of 105 Holstein cows within the first 30 DIM, separated by parity.

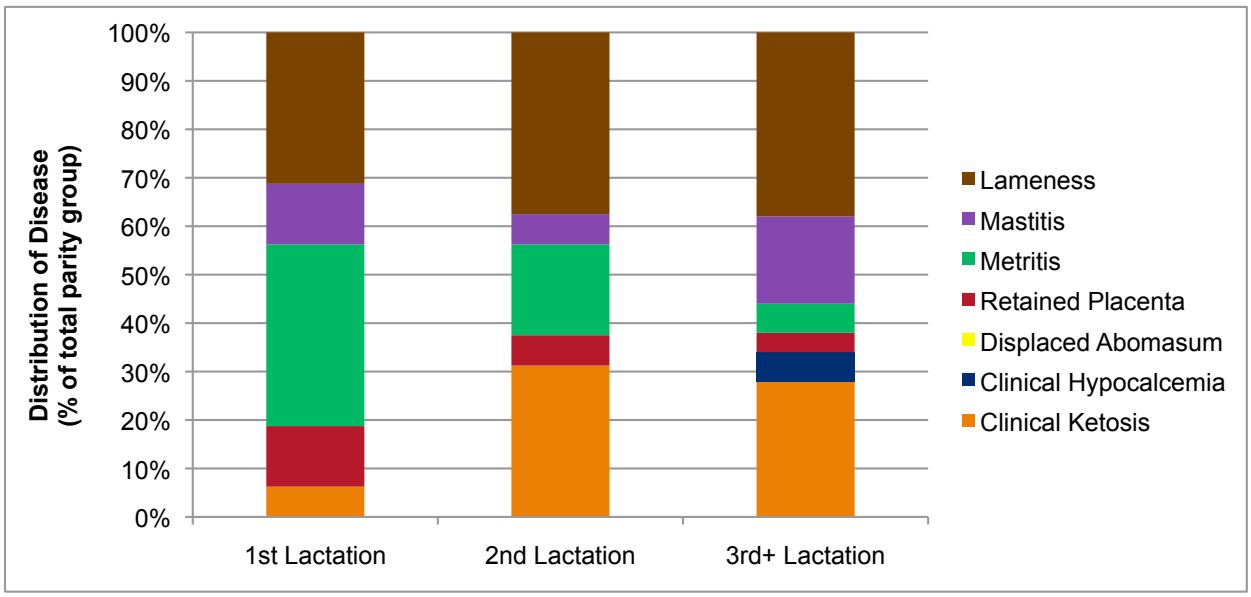

Figure 8. The percent distribution of disease conditions (clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and/or metritis) among the study population of 105 Holstein cows within the first 30 DIM, separated by parity.

Associations: Rate of Body Weight Change

All cows had a negative change in body weight over the course of the study (i.e., the first 30 DIM). The analyses for change in body weight were stratified by parity (parity = 1, parity = 2, and parity ≥ 3) because the change in body weight was different among these groups ($p < 0.01$), as shown in Figure 9. Tables 3A-3D show the effect on body weight change based on the interaction between normal or abnormal values for each indicator metabolite and the presence or absence of disease: prepartum NEFA (3A), postpartum NEFA (3B), BHB (3C) and calcium (3D). When an interaction term was included, the effect of only the blood metabolite or disease occurrence could not be evaluated individually because the result depends on the values of both terms in the interaction. Therefore, the interaction estimates and p-values are reported.

A more precipitous weight loss during the first 30 DIM within all three parity groups were observed in cows which developed disease. For heifers, having a high prepartum NEFA value exacerbated this weight loss. Second lactation cows, having a high BHB reading within the first week following parturition was associated with accelerated weight loss. For third lactation and greater cows, having a high postpartum NEFA value was associated with more rapid weight loss throughout the first 30 DIM.

However, other metabolite readings and their association with the rate of weight loss is less clear. For example, second lactation cows with normal prepartum NEFA levels and no disease lost weight more quickly than those which developed disease. Another surprising result was that heifers and second-lactation cows with a high postpartum NEFA or high BHB levels and disease demonstrated the slowest rate of weight loss. Heifers with disease but normal NEFA or BHB readings lost weight more quickly than their healthy peers. Finally, third lactation and greater cows with both high prepartum NEFA values and disease lost weight more quickly

than healthy individuals, however, cows that experienced only one condition or the other demonstrated a reduced rate of weight loss. Associations were inconsistent between calcium status and weight loss. Occasionally the cows that experienced the most rapid weight loss were hypocalcemic and had disease, or had only one of these conditions.

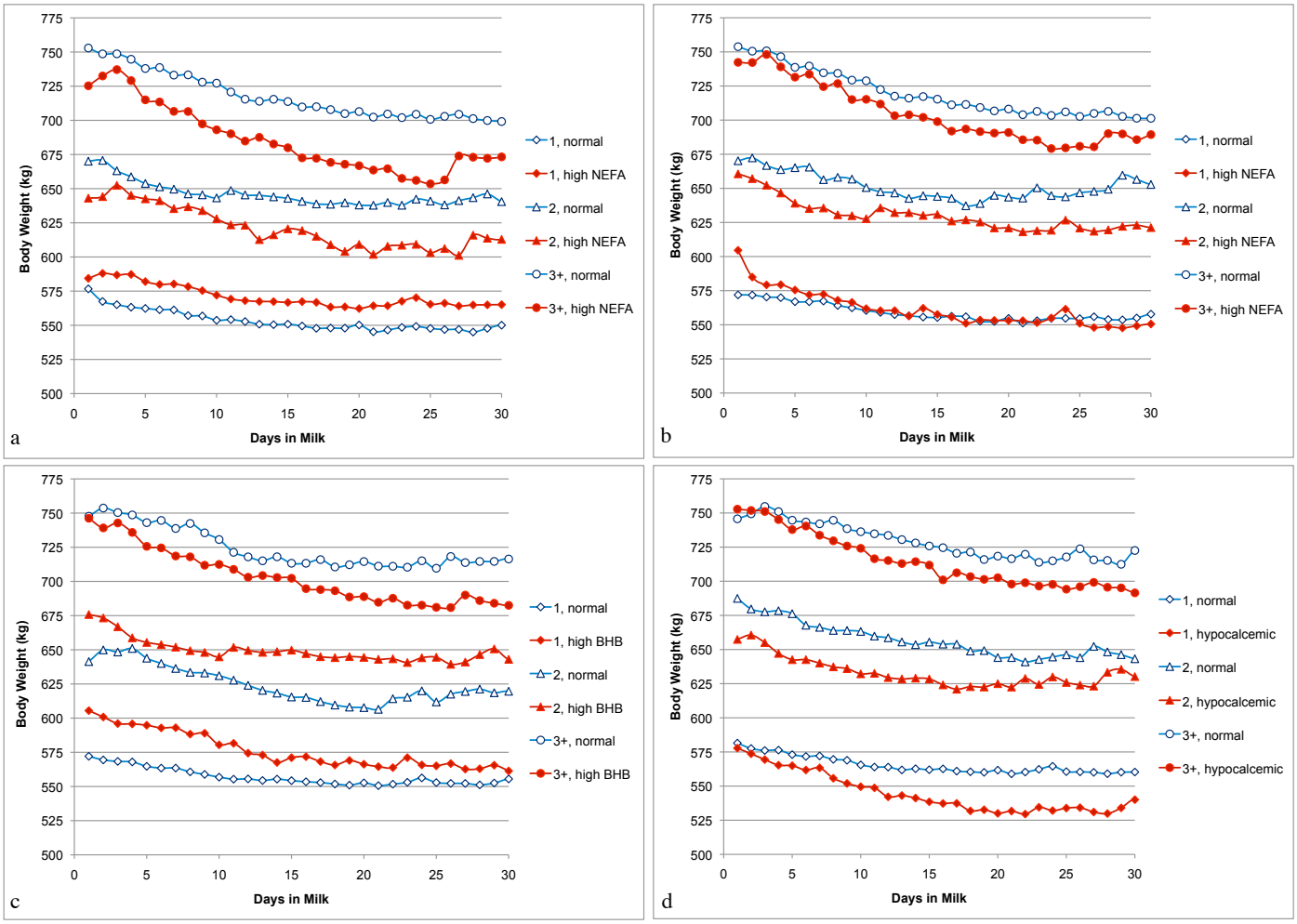

Figure 9. Average body weight in kilograms of the study population of 105 Holstein cows within the first 30 DIM, separated by parity and:

a A prepartum NEFA of $\geq$ 0.3 mEq/dL and/or

b A postpartum NEFA of $\geq$ 0.7 mEq/dL.

c Any BHB reading of $\geq$ 1.2 mmol/L.

d Any calcium reading of $\geq$ 6.0 mg/dL to 8.0 mg/dL.

Starting body weight and the slope of body weight was different among parity groups ($p < 0.01$).

Parity	Interaction Terms	Estimate	S.E.	D.F.	P-value[b]
1st	Normal NEFA, no disease	-2.0	0.1	1045	**< 0.0001**
	Normal NEFA, disease	-3.0	0.1	1045	**< 0.0001**
	High NEFA, no disease	-2.1	0.1	1045	**< 0.0001**
	High NEFA, disease	-2.5	0.1	1045	**< 0.0001**
2nd	Normal NEFA, no disease	-3.5 [c]	0.2	866	**< 0.0001**
	Normal NEFA, disease	-2.8	0.2	866	**< 0.0001**
	High NEFA, no disease	-3.3 [c]	0.2	866	**< 0.0001**
	High NEFA, disease	.	.	.	**.**
3rd+	Normal NEFA, no disease	-1.7	0.1	1285	**< 0.0001**
	Normal NEFA, disease	-1.5	< 0.1	1285	**< 0.0001**
	High NEFA, no disease	-1.0	0.2	1285	**< 0.0001**
	High NEFA, disease	-2.9	0.1	1285	**< 0.0001**

Table 3A. The Least Squares Means estimate of the interaction between prepartum NEFA of ≥ 0.3 mEq/dL and dichotomized disease[a] on body weight change in 105 Holstein cows within the first 30 DIM, separated by parity.

[a] Diseases include clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and/or metritis. Note that each condition is not mutually exclusive.

[b] The p-value from the Least Squares Means test evaluates whether the interaction estimate is different from zero. This p-value is reported if all interaction combinations were populated. If any interaction combinations were missing (indicated by point marks), the p-value for the Type 3 Test of Mixed Effects, part of the PROC MIXED analysis, is reported.

[c] Estimates with the same superscript were not statistically different from each other ($p > 0.05$).

Parity	Interaction Terms	Estimate	S.E.	D.F.	P-value[b]
1st	Normal NEFA, no disease	-2.4	0.1	1045	**< 0.0001**
	Normal NEFA, disease	-3.2	0.1	1045	**< 0.0001**
	High NEFA, no disease	-2.1	0.1	1045	**< 0.0001**
	High NEFA, disease	-1.7	0.2	1045	**< 0.0001**
2nd	Normal NEFA, no disease	-2.4 [c]	0.2	865	**< 0.0001**
	Normal NEFA, disease	-2.6 [c]	0.2	865	**< 0.0001**
	High NEFA, no disease	-3.4	0.2	865	**< 0.0001**
	High NEFA, disease	-2.0	0.2	865	**< 0.0001**
3rd+	Normal NEFA, no disease	-1.7 [d]	0.1	1285	**< 0.05**
	Normal NEFA, disease	-1.9 [d]	0.1	1285	**< 0.05**
	High NEFA, no disease	-2.1	0.1	1285	**< 0.05**
	High NEFA, disease	-2.6	0.1	1285	**< 0.05**

Table 3B. The Least Squares Means estimate of the interaction between postpartum NEFA of ≥ 0.7 mEq/dL and dichotomized disease[a] on body weight change in 105 Holstein cows within the first 30 DIM, separated by parity.

[a] Diseases include clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and/or metritis. Note that each condition is not mutually exclusive.

[b] The p-value from the Least Squares Means test evaluates whether the interaction estimate is different from zero.

[c-d] Estimates with the same superscript were not statistically different from each other ($p > 0.05$)

Parity	Interaction Terms	Estimate	S.E.	D.F.	P-value [b]
1st: Sample 1 or 2	Normal BHB, no disease	-2.3	0.1	1046	**< 0.0001**
	Normal BHB, disease	-3.3	0.1	1046	**< 0.0001**
	High BHB, no disease	.	.	.	**.**
	High BHB, disease	-1.9	0.1	1046	**< 0.0001**
2nd: Sample 1 or 2	Normal BHB, no disease	-3.3	0.2	865	**< 0.001**
	Normal BHB, disease	-2.9	0.2	865	**< 0.001**
	High BHB, no disease	-3.8	0.2	865	**< 0.001**
	High BHB, disease	-2.6	0.2	865	**< 0.001**
3rd+: Sample 1 or 2	Normal BHB, no disease	-1.6 [c]	0.1	1285	**< 0.0001**
	Normal BHB, disease	-1.4 [d]	0.1	1285	**< 0.0001**
	High BHB, no disease	-1.6 [c,d]	0.1	1285	**< 0.0001**
	High BHB, disease	-2.4	0.1	1285	**< 0.0001**

Table 3C. The Least Squares Means estimate of the interaction between BHB of ≥ 1.2 mmol/L and dichotomized disease[a] on body weight change in 105 Holstein cows within the first 30 DIM, separated by parity.

[a] Diseases include clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and/or metritis. Note that each condition is not mutually exclusive.

[b] The p-value from the Least Squares Means test evaluates whether the interaction estimate is different from zero. This p-value is reported if all interaction combinations were populated. If any interaction combinations were missing (indicated by point marks), the p-value for the Type 3 Test of Mixed Effects, part of the PROC MIXED analysis, is reported.

[c-d] Estimates with the same superscript were not statistically different from each other ($p > 0.05$)

Parity	Interaction Terms	Estimate	S.E.	D.F.	P-value [b]
1st: Sample 2	Normal calcium, no disease	-1.7 [c]	0.1	1045	**< 0.0001**
	Normal calcium, disease	-2.7	0.1	1045	**< 0.0001**
	Hypocalcemic, no disease	-3.2	0.1	1045	**< 0.0001**
	Hypocalcemic, disease	-1.8 [c]	0.1	1045	**< 0.0001**
2nd: Any sample	Normal calcium, no disease	-3.9 [d]	0.2	865	**< 0.0001**
	Normal calcium, disease	-4.6	0.2	865	**< 0.0001**
	Hypocalcemic, no disease	-3.8 [d]	0.2	865	**< 0.0001**
	Hypocalcemic, disease	-2.8	0.2	865	**< 0.0001**
3rd+: Sample 2	Normal calcium, no disease	-1.8 [e,f]	0.2	1285	**< 0.005**
	Normal calcium, disease	-1.7 [e]	0.1	1285	**< 0.005**
	Hypocalcemic, no disease	-2.0 [f]	0.1	1285	**< 0.005**
	Hypocalcemic, disease	-2.5	0.1	1285	**< 0.005**

Table 3D. The Least Squares Means estimate of the interaction between calcium of ≥ 0.6 to 0.8 mg/dL and dichotomized disease[a] on body weight change in 105 Holstein cows within the first 30 DIM, separated by parity.

[a] Diseases include clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and/or metritis. Note that each condition is not mutually exclusive.

[b] The p-value from the Least Squares Means test evaluates whether the interaction estimate is different from zero.

[c-f] Estimates with the same superscript were not statistically different from each other ($p > 0.05$)

Associations: Milk Yield

There was no statistically significant difference between milk production in the 2nd and ≥ 3rd lactation ($p > 0.05$), as shown in Figure 10. However, there was a significant difference in milk production between 1st lactation and ≥ 2nd lactation cows ($p < 0.01$). Therefore, the milk production analysis was stratified by parity in two groups (parity = 1 and parity ≥ 2). Tables 4A-4D show the effect of total milk production within 30 DIM based on the interaction between normal or abnormal values for each indicator metabolite and the presence or absence of disease: prepartum NEFA (4A), postpartum NEFA (4B), BHB (4C) and calcium (4D). When an interaction term was included, the effect of only the blood metabolite or disease occurrence could not be evaluated individually because the result depends on the values of both terms in the interaction. Therefore, the interaction estimates and p-values are reported.

Heifers with elevated prepartum NEFA readings had the highest milk yield, a result that was not expected. Prepartum NEFA otherwise impacted lactation as expected: both disease and a high NEFA value were independently associated with a decreased milk yield, but the greatest loss in milk yield occurred in cows that experienced both conditions.

Cows within all parity groups that presented with high postpartum NEFA readings and disease during the course of the study had the lowest milk yield, while second and greater lactation cows that were unaffected with either condition had the highest yield. However, the impact of either elevated prepartum NEFA or disease alone, generated statistically similar, slight reductions in milk yield.

Sampling BHBs both day three and five and looking at the presence of disease was useful in predicting milk yield in second lactation and greater cows. As shown in Table 4C, if sampling resources are limited, little of that resolution is lost by only sampling on day three.

Normocalcemic second and greater lactation cows had the highest milk yield. Unexpectedly, hypocalcemic heifers produced substantially more milk compared to those with normal calcium values. Excluding this anomaly, both sub-clinical hypocalcemia and disease reduced milk production in all cows in the study, but the combination was more potent.

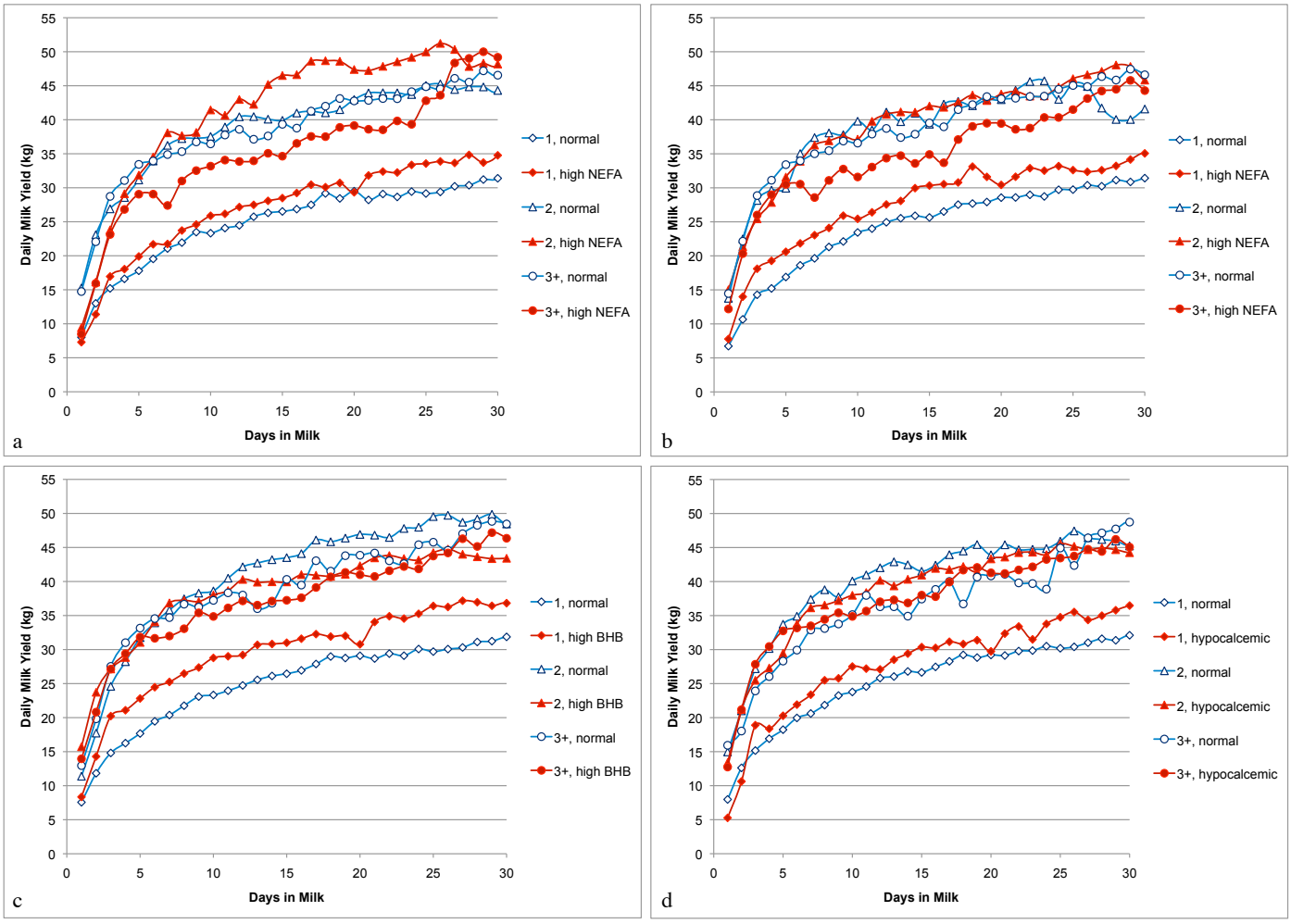

Figure 10. Average daily milk yield in kilograms of the study population of 105 Holstein cows within the first 30 DIM, separated by parity and:

a A prepartum NEFA of ≥ 0.3 mEq/dL and/or

b A postpartum NEFA of ≥ 0.7 mEq/dL.

c Any BHB reading of ≥ 1.2 mmol/L.

d Any calcium reading of ≥ 6.0 mg/dL to 8.0 mg/dL.

There was no significant difference in milk yield between parity groups 2 and ≥ 3 ($p > 0.05$), but there was a difference between parity groups 1 and ≥ 2 ($p < 0.01$).

Parity	Interaction Terms	Estimate	S.E.	D.F.	P-value[b]
1st	Normal NEFA, no disease	972 [c]	18.5	1045	**< 0.0001**
	Normal NEFA, disease	954 [c,d]	19.4	1045	**< 0.0001**
	High NEFA, no disease	1056	17.8	1045	**< 0.0001**
	High NEFA, disease	938 [d]	20.2	1045	**< 0.0001**
2nd+	Normal NEFA, no disease	1229	15.7	2155	**< 0.05**
	Normal NEFA, disease	1128 [e]	15.6	2155	**< 0.05**
	High NEFA, no disease	1113 [e]	19.1	2155	**< 0.05**
	High NEFA, disease	1063	17.9	2155	**< 0.05**

Table 4A. The Least Squares Means estimate of the interaction between prepartum NEFA of ≥ 0.3 mEq/dL and dichotomized disease[a] on total milk yield in 105 Holstein cows within the first 30 DIM, separated by parity.

[a] Diseases include clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and/or metritis. Note that each condition is not mutually exclusive.

[b] The p-value from the Least Squares Means test evaluates whether the interaction estimate is different from zero.

[c-e] Estimates with the same superscript were not statistically different from each other ($p > 0.05$)

Parity	Interaction Terms	Estimate	S.E.	D.F.	P-value [b]
1st	Normal NEFA, no disease	1064	19.1	1045	**0.6**
	Normal NEFA, disease	994 [c]	19.5	1045	**0.6**
	High NEFA, no disease	992 [c]	20.0	1045	**0.6**
	High NEFA, disease	906	30.0	1045	**0.6**
2nd+	Normal NEFA, no disease	1205	18.3	2155	**0.5**
	Normal NEFA, disease	1113 [d]	17.0	2155	**0.5**
	High NEFA, no disease	1127 [d]	17.6	2155	**0.5**
	High NEFA, disease	1050	18.9	2155	**0.5**

Table 4B. The Least Squares Means estimate of the interaction between postpartum NEFA of ≥ 0.7 mEq/dL and dichotomized disease[a] on total milk yield in 105 Holstein cows within the first 30 DIM, separated by parity.

[a] Diseases include clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and/or metritis. Note that each condition is not mutually exclusive.

[b] The p-value from the Least Squares Means test evaluates whether the interaction estimate is different from zero.

[c-d] Estimates with the same superscript were not statistically different from each other ($p > 0.05$)

Parity	Interaction Terms	Estimate	S.E.	D.F.	P-value[b]
1st: Sample 1 or 2	Normal BHB, no disease	1033	18.0	1046	**< 0.0001**
	Normal BHB, disease	983	19.7	1046	**< 0.0001**
	High BHB, no disease	.	.	.	**.**
	High BHB, disease	916	23.4	1046	**< 0.0001**
2nd+: Sample 1 only	Normal BHB, no disease	1262	7.1	2155	**< 0.0001**
	Normal BHB, disease	1144	6.6	2155	**< 0.0001**
	High BHB, no disease	899	21.2	2155	**< 0.0001**
	High BHB, disease	1116	11.1	2155	**< 0.0001**
2nd+: Sample 1 or 2	Normal BHB, no disease	1243	14.3	2155	**< 0.0001**
	Normal BHB, disease	1127 [c]	15.2	2155	**< 0.0001**
	High BHB, no disease	1067	19.1	2155	**< 0.0001**
	High BHB, disease	1120 [c]	14.1	2155	**< 0.0001**

Table 4C. The Least Squares Means estimate of the interaction between BHB of ≥ 1.2 mmol/L and dichotomized disease[a] on total milk yield in 105 Holstein cows within the first 30 DIM, separated by parity.

[a] Diseases include clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and/or metritis. Note that each condition is not mutually exclusive.

[b] The p-value from the Least Squares Means test evaluates whether the interaction estimate is different from zero. This p-value is reported if all interaction combinations were populated. If any interaction combinations were missing (indicated by point marks), the p-value for the Type 3 Test of Mixed Effects, part of the PROC MIXED analysis, is reported.

[c] Estimates with the same superscript were not statistically different from each other ($p > 0.05$).

Parity	Interaction Terms	Estimate	S.E.	D.F.	P-value [b]
1st: Any sample	Normal calcium, no disease	954 [c]	16.9	1045	**< 0.0001**
	Normal calcium, disease	953 [c]	17.1	1045	**< 0.0001**
	Hypocalcemic, no disease	1119	17.6	1045	**< 0.0001**
	Hypocalcemic, disease	889	23.2	1045	**< 0.0001**
2nd+: Sample 3 only	Normal calcium, no disease	1206	17.0	2155	**< 0.0001**
	Normal calcium, disease	1086 [d,e]	17.0	2155	**< 0.0001**
	Hypocalcemic, no disease	1100 [d,f]	19.3	2155	**< 0.0001**
	Hypocalcemic, disease	1078 [e,f]	18.2	2155	**< 0.0001**

Table 4D. The Least Squares Means estimate of the interaction between calcium of ≥ 6.0 to 8.0 mg/dL and dichotomized disease[a] on total milk yield in 105 Holstein cows within the first 30 DIM, separated by parity.

[a] Diseases include clinical ketosis, displaced abomasum, lameness, clinical mastitis, retained placenta, and/or metritis. Note that each condition is not mutually exclusive.

[b] The p-value from the Least Squares Means test evaluates whether the interaction estimate is different from zero.

[c-f] Estimates with the same superscript were not statistically different from each other ($p > 0.05$)

Discussion

A practical goal of this research is to reliably identify early warning signs that a cow is at high risk of experiencing a precipitous loss in body weight and/or milk yield, so that therapeutic intervention can be administered. Negative energy balance and sub-clinical hypocalcemia are of interest because of their implications for economic return and animal welfare. They are of particular interest in the manifestation of and interactions with disease, because health problems tend to occur not in isolation, but as part of a physiological complex (Fleischer et al., 2001; Lean, 2011). Metabolic indicators such as NEFA, BHB, and calcium measurements are useful for monitoring the cow's current homeorhetic status, and in some cases are also associated with the development of disease (DeGaris and Lean, 2008; McArt et al., 2013; Suthar et al., 2013). Disease has an important impact on overall bovine health and consequential economic return (Duffield et al., 2009; Mulligan and Doherty, 2008; Rajala-Schultz et al., 1999; Thirunavukkarasu et al., 2010). In this study, measurements were made early in the postpartum period on transition period cows to find the best predictive blood metabolite criteria that was associated with body weight change and milk yield with disease as a covariate and while controlling for herd as a random effect.

Cows from all three parity groups which developed disease had a higher rate of weight loss and lower milk yield during the first 30 DIM. Third and greater parity cows experienced an overall higher disease incidence than second or lower parity cows, a finding supported by previous studies (Gallo et al., 1996; Lean, 2011; McArt et al., 2013; Reinhardt et al., 2011). Among these conditions, lameness and ketosis were the most prevalent diseases experienced by second and third and greater lactation cows, while metritis was the most common disorder

experienced by heifers. All heifers with high BHB readings at three and five DIM were developed disease, as did all second lactation cows with high prepartum NEFA. In future work, disease development itself could be analyzed as an undesirable outcome, rather than simply as a predictor.

In each parity group, a different indicator metabolite predicted rate of weight change: prepartum NEFA was the best predictor for heifers, a high day three or day five BHB reading was predictive for second lactation cows, and postpartum NEFA values were most useful for third and greater lactation cows. Calcium was not a reliable indicator for a cow's change in body weight for the first 30 DIM for any lactation group.

Prepartum NEFA and day three and day five BHB measurements were reliable indicators of milk yield in second lactation and greater cows. For heifers, abnormal metabolite values significantly impacted milk yield when it was concurrent with disease. One unusual finding was that heifers with high prepartum NEFA values or sub-clinical hypocalcemia which did not develop disease produced significantly more milk than their unaffected herd mates. Chapinal et al. (2012) discovered that cows with high prepartum NEFA and low calcium values had a reduced milk yield. However, Østergaard and Larsen (2000) found that sub-clinical hypocalcemia at calving does not affect milk yield, and Jawor et al. (2012) reported that cows with sub-clinical hypocalcemia actually produced more milk, although only third and greater parity cows sustained this higher yield through 280 DIM and this result was presented without the effects of disease.

From the perspective of input costs, BHB is the least expensive test to run at only $1.30 compared to the cost of calcium at $8.00 and NEFA at $11.00, but these costs are almost

negligible when these prices are put into the perspective of risking disease and a loss in milk production due to negative energy balance and sub-clinical hypocalcemia. As a review of relevant literature has revealed, disease development can cost a farmer up to hundreds of dollars from a single cow, a price which reflects the cost of medical treatment, lost milk production, and in bringing the cow back to a healthy status or replacing it if it has to be culled. As demonstrated in this study, these metabolites and disease can affect a cow's milk yield during the first 30 DIM. Even if affected cows only lose 100 kg of milk during the transition period, United States Department of Agriculture statistics indicate a converted market price of approximately $44 (USDA, 2013), which is significant if multiple cows are affected as they were in this study.

In this study, back fat and body condition score were only weakly correlated with one another. Without the ability to rank the validity of one measurement over the other, neither was analyzed as an outcome. However, as they could be indicators of disease development in dairy cattle (Lassen et al., 2003; Roche et al., 2009), these are measurements of interest. Future studies could determine how these measurements are correlated with the cow's overall body weight and their association with blood metabolites, milk yield, and disease.

The greatest limiting factor of this study was the size of the sample population. There were not enough cows to represent every possible interaction between normal versus deviant blood metabolite levels and presence of disease to comprehensively analyze their relationships with rate of weight loss and milk production. While this is indicative of good animal health and management on the participant farms in this study, it limited our statistical analyses.

In light of these results, a useful management strategy for minimizing the rate of weight loss and maximizing milk yield during the first 30 DIM is to conduct different metabolite tests

for cows depending on their parity, and to remain vigilant in observing cows for potential disease development. As previously mentioned, there are outside costs and minor labor inconveniences in collecting samples for testing. However, if high risk cows can be identified early during the lactation period, intervention can be initiated to maintain energy balance and calcium homeostasis, and assist in the prevention of disease. By maximizing individual cow health, the dairy producer benefits financially and optimizes the health and welfare of the herd as a whole.

Conclusion

The objectives in this study were to help identify high risk cows, explain which factors predispose them to negative outcomes, and make economically sound, practical recommendations for farmers to improve herd monitoring and management. Through the analysis of blood metabolite tests and disease and their impact on rate of weight loss and milk yield, certain metabolites were recognized as reliable indicators depending on the outcome of interest, the cow's parity, and whether abnormal metabolite readings coincided with the development of disease during the first 30 DIM.

A dairy producer can use measurements of prepartum or day five NEFA, and day three and day five BHB to screen cows according to parity and whether the outcome of interest for that cow is change in body weight or milk yield during the first 30 DIM. Serum calcium levels are not reliable predictors of a change in body weight, and only affect milk yield when sub-clinical hypocalcemia occurs simultaneously with disease. By identifying high-risk cows, early intervention protocols can prevent the loss of milk, minimize animal welfare concerns, and maximize economic sustainability for the dairy producer.

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